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Nickel

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12.1 Introduction and History

Historically, the first study of the biological action of nickel was reported in 1826 when the oral nickel toxicity signs exhibited by rabbits and dogs were described. Between 1853 and 1912 numerous other studies on the pharmacologic and toxicologic actions of various nickel compounds were described. The findings from these studies were summarized by Nriagu (1980a). The first reports on the presence of nickel in plant and animal tissues appeared in 1925 (Berg, 1925; Bertrand and Macheboeuf, 1925). Although Bertrand and Nakamura (1936) first suggested that nickel may be an essential element, conclusive evidence for essentiality did not appear until 1970–1975. Thus, most of the studies on the biochemical, nutritional, and physiological roles of nickel were done subsequent to 1970.

12.2 Nickel and Its Compounds in Cells and Tissues

Divalent nickel is apparently the important oxidation state of nickel in biochemistry. Divalent nickel forms a large number of complexes encompassing coordination numbers 4, 5, and 6, and all main structural types, which include square planar, square pyramidal, tetrahedral, octahedral, and trigonal pyramidal. Moreover, in complicated equilibria Ni^{2+} complexes often exist between these structural types. Like other ions of the first transition series, Ni^{2+} has the ability

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to complex, chelate, or bind with many substances of biological interest. Thus, it is not surprising that nickel is a ubiquitous element found in all biological material. Various authors have tabulated the nickel content in numerous plants, microorganisms, and animals in a recently published book (Nriagu, 1980b). Except for some nickel-accumulating plants and marine species, nickel levels in nearly all biological materials are in the range of nanograms per gram (ppb) to a few micrograms per gram (ppm).

The binding of nickel *in vitro* to numerous molecules isolated from cellular materials may have important counterparts *in vivo*. Sunderman (1977) suggested that ultrafilterable Ni^{2+} binding ligands play an important role in extracellular transport of nickel, intracellular binding of nickel, and excretion of nickel in urine and bile. In human serum, amino acids were found to be components of the low-molecular-weight Ni^{2+}-binding fraction and L-histidine was found to be the main Ni^{2+}-binding amino acid (Lucassen and Sarkar, 1979). At physiological pH, nickel coordinates with histidine via the imidazole nitrogen. In rabbit serum, cysteine, histidine, and aspartic acid may be involved in the binding of Ni^{2+} (Sunderman, 1977). The binding of Ni^{2+} by cysteine probably occurs at the N (amino) and S (sulfhydryl) sites. Computer approaches have predicted that the predominant interaction with naturally occurring low-molecular-weight ligands would occur with histidine and cysteine (Jones *et al.*, 1980).

The binding of nickel by some proteins has been suggested to be of physiological significance. Albumin is the principal Ni^{2+}-binding protein in human, bovine, rabbit, and rat serum. Nickel apparently is bound to albumin by a square planar ring formed by the terminal amino group, the first two peptide nitrogen atoms at the N-terminus, and the imidazole nitrogen of the histidine residue which is located at the third position from the N-terminus. Canine and porcine albumins, which contain tyrosine instead of histidine at the third position, have less affinity for Ni^{2+} than albumins from other species (Sunderman, 1977). Another serum protein, histidine-rich glycoprotein (HRG), apparently has the ability to bind Ni^{2+} (Morgan, 1981). Both albumin and HRG have been suggested to have a role in the transport and homeostasis of nickel in serum. Another histidine-rich protein, purified from newborn rat epidermis, also binds Ni^{2+}. Takeda *et al.* (1981) suggested that in addition to contributing to the formation of the matrix material of cornified cells, the histidine-rich protein regulates the utilization of trace metals, including nickel, in granular cells. They also suggested that the carboxyl groups of aspartic acid and glutamic acid are more likely to be involved in the binding of Ni^{2+} than histidine in the epidermal histidine-rich protein.

The binding of nickel to nucleotides also may be of physiological significance. Nickel, via a structural role, may be a stabilizer for DNA and RNA. This role may be more important in some species than others. Nickel was found in

relatively high amounts in chromatin of dinoflagellates (Kearns and Sigee, 1980). In these organisms, metal ions, including nickel, may be of particular importance for chromatin structure because the chromatin is permanently condensed with no associated histones. Although the phosphate oxygens are a major binding site for nickel, there is considerable evidence that Ni^{2+} interacts with the purine base adenine at the N-7 site in the nucleotide ATP.

Organic acids apparently are major bioligands in the uptake and translocation of nickel in plants. Nickel translocates in plants as a stable anionic organic complex. Early suggestions were that these complexes were formed by amino acids. However, recent phytochemical studies showed that in nickel-accumulating plants, nickel was contained as anionic citrate or malate complexes (Kersten *et al.*, 1980).

Nickel is an integral component, not just bound, to some biological substances. To date, four nickel-containing substances have been, or are tentatively, identified.

A nickel-containing macroglobulin, nickeloplasmin, has been found in human and rabbit serum (Sunderman, 1977). Characteristics of nickeloplasmin include an estimated molecular weight of 7.0×10^5, nickel content of 0.90 g-at/mol, and esterolytic activity. Nickel in nickeloplasmin is not readily exchangeable with $^{63}Ni^{2+}$ *in vivo* or *in vitro*. Nickeloplasmin has been suggested to be a ternary complex of serum α_1-macroglobulin with a nickel constituent of serum. Sunderman (1977) noted that a 9.55 α_1-glycoprotein that strongly bound Ni^{2+} has been isolated from human serum and thus suggested that nickeloplasmin might represent a complex of the 9.55 α_1-glycoprotein with serum α_1-macroglobulin. Unfortunately, there is no clear indication of the physiological significance or function of nickeloplasmin.

Urease from several plants and microorganisms was found to be a nickel metalloenzyme. Urease from the jack bean *(Canavalia ensiformis)* was the first example of this natural nickel metalloenzyme. Jack bean urease (E.C. 3.5.1.5) contains stoichiometric amounts of nickel, 2.00 ± 0.12 g-at/mol of 96,600-dalton subunits (Dixon *et al.*, 1980a,b). Nickel in jack bean urease is part of the active site and is tightly bound, being similar to the zinc ion in yeast alcohol dehydrogenase (E.C. 1.1.1.1) and manganous ion in chicken liver pyruvate carboxylase (E.C. 6.4.1.1). Jack bean urease is stable and fully active in the presence of 0.5 m*M* EDTA at neutral pH. The nickel ion can be removed only upon exhaustive dialysis in the presence of chelating agents or by EDTA at low pH, and then it is not possible to restore nickel with reconstitution of enzymatic activity. The findings of Dixon *et al.* (1980b) were consistent with an octahedral coordination of Ni^{2+} with urease as seen in model complexes and Ni^{2+}-phosphoglucomutase and Ni^{2+}-carboxypeptidase. Jack bean urease has relatively low reactivity of the active-site sulfhydryl groups, thus suggesting coordination of

the active-site nickel with the unreactive cysteine. Of interest, jack bean urease was the first enzyme protein to be crystallized. It took 50 years to show that this enzyme contains nickel in its structure.

More than 50% of the nickel taken up by methanogenic bacteria is incorporated into a low-molecular-weight compound with an absorption maximum at 430 nm (Thauer *et al.*, 1980). This nickel-containing compound, which has been found in every methanogenic bacterium examined to date, has been named factor F_{430}. Preliminary experiments indicate that nickel is tightly bound to factor F_{430} because $^{63}Ni^{2+}$ is not exchanged with factor F_{430} nickel even when the incubation mixture is 6 *N* HCl. The exact structure of factor F_{430}, which has a mass per mol nickel of approximately 1500 daltons, has not been elucidated. However, findings to date suggest that factor F_{430} contains a nickel tetrapyrrole structure. A nickel-containing degradation product of factor F_{430} has an absorption spectrum in the visible region resembling that of vitamin B_{12}. Also, biosynthetic studies indicate that, per mol of nickel, 8 mol of δ-aminolevulinic acid are incorporated into factor F_{430}. Like nickeloplasmin, there is no clear indication as to the physiological significance or function of factor F_{430}.

In acetogenic bacteria, the reductive carboxylation of methyl tetrahydrofolate to acetate is catalyzed by a multienzyme complex with one portion having carbon monoxide dehydrogenase activity. The synthesis of the moiety with carbon monoxide dehydrogenase activity requires nickel (Thauer *et al.*, 1980). Furthermore, analyses of the multienzyme complex indicate that the moiety is a protein with a nickel-containing prosthetic group with properties similar to those of vitamin B_{12}.

12.3 Nickel Deficiency

For animals, the first description of possible signs of nickel deprivation appeared in 1970. However, those findings, and others that followed shortly thereafter, were obtained under conditions that produced suboptimal growth in the experimental animals (Nielsen, 1980a). Also, some reported signs of nickel deprivation appeared inconsistent. However, retrospective analyses of the methodology used in those studies indicated that much of the inconsistency was probably caused by variance in the iron status of, and environmental conditions for, the experimental animals. Thus, most of the early findings were true nickel deprivation signs in animals under certain dietary and environmental conditions. The importance of iron in nickel nutrition will be described *(vide infra)*.

Since 1975, diets and environments that allow for apparently optimal growth and survival of experimental animals have been used in the study of nickel metabolism and nutrition. To date, signs of nickel deprivation have been described for six animal species—chick, cow, goat, minipig, rat, and sheep.

Nielsen (1980a) summarized the signs of nickel deprivation in chicks. The signs included depressed hematocrits, oxidative ability of the liver in the presence of α-glycerophosphate and yellow lipochrome pigments in the shank skin, and ultrastructural abnormalities in the liver. The abnormalities were characterized by pyknotic nuclei and dilated cisternal lumens of the rough endoplasmic reticulum. The cisternae appeared to be draped around the mitochondria, bringing the ribosomes into close proximity to the outer mitochondrial membrane. There was a general impression that the ribosomes were more irregular in their spacing on the rough endoplasmic reticulum and that large amounts of membrane were devoid of ribosomes. Mitochondria were numerous and closely packed and elongate forms were common. The matrices of the mitochondria were reduced in density and appeared hydrated.

Two independent laboratories have described signs of nickel deprivation for the rat. In the laboratory of Nielsen (1980a), successive generations of rats were exposed to a low-nickel diet throughout fetal, neonatal, and adult life. Signs of nickel deprivation found in the rat included elevated perinatal mortality, unthriftiness characterized by a rough coat and/or uneven hair development in pups, pale livers, and ultrastructural changes in the liver. The most obvious effect of nickel deprivation on liver ultrastructure was a reduced amount of rough endoplasmic reticulum which appeared disorganized in that the normal "stacking" of the cisternae was partially or totally absent. Nickel deprivation appeared to depress growth and hematocrits of rats in these experiments in which an adequate, relatively available form of dietary iron was used, but these signs were not consistently significant. Subsequent to those studies, Nielsen (1980a) found that the iron status of the rat has a major influence on the extent and severity of the signs of nickel deprivation. Thus, when relatively unavailable ferric sulfate at marginally adequate levels was the dietary source of iron, depressed growth and hematopoiesis were consistently found in nickel-deprived offspring. Furthermore, Nielsen (1980a) found that when the iron content and form of the diet was properly manipulated, nickel deprivation could be induced in rats that were not from nickel-deprived dams. Apparent signs of deficiency in weanling rats fed a diet deficient in nickel and marginally adequate in levels of relatively unavailable ferric sulfate for 9–11 weeks included depressed hematopoiesis and liver iron, and elevated plasma lipids and liver copper.

In a series of reports summarized recently, Kirchgessner and Schnegg (1980) described the following nickel deprivation signs for offspring of nickel-deprived dams. At age 30 days, nickel-deprived pups exhibited significantly depressed growth, hematocrit, hemoglobin, and erythrocyte counts; those signs were still evident, but less marked, at age 50 days. However, at age 120 days, depressed growth and hematopoiesis were not evident in the nickel-deprived offspring. Thus, like those of Nielsen (1980a), the findings on growth and hematopoiesis were inconsistent. Perhaps the findings of Kirchgessner and Schnegg (1980)

were also influenced by a changing iron status of the animal. Although the initial form of iron used in their studies was ferrous sulfate, the method of adding the ferrous sulfate to the diet could have converted some Fe^{2+} to Fe^{3+}, possibly in varying amounts for each diet. The ferrous sulfate was put into distilled water with several other mineral salts, then added to a moist diet mixture, and heated at 50° to remove water. Also, the diet may have contained residual EDTA (used to prepare the casein), which can affect the availability of iron. Kirchgessner and Schnegg (1980) found that the severity of the nickel deprivation signs in young pups was reduced by increasing dietary iron from 50 μg/g to 100 μg/g.

Other apparent nickel deprivation signs in the young pups described by Kirchgessner and Schnegg (1980) included: (1) At age 30 days, the activities of the liver enzymes malate dehydrogenase, glucose-6-phosphate dehydrogenase, isocitrate dehydrogenase, lactate dehydrogenase, glutamate-oxalacetate transaminase, and glutamate-pyruvate transaminase were depressed whereas the activities of alkaline phosphatase and creatine kinase were elevated. At age 50 days, nickel deprivation did not significantly affect liver malate dehydrogenase, or glucose-6-phosphate dehydrogenase activity. (2) At age 30 days, the activities of the kidney enzymes glutamate dehydrogenase, glutamate-oxalacetate transaminase, and glutamate-pyruvate transaminase were depressed. (3) The levels of urea, ATP, and glucose in serum were reduced. (4) The levels of triglycerides, glucose, and glycogen in liver were reduced. (5) Iron absorption was impaired. (6) Levels of iron, copper, and zinc were depressed in the liver, kidney, and spleen. (7) Levels of calcium and phosphorus were depressed and the level of magnesium was elevated in the femur. (8) Activities of proteinase and leucine arylamidase increased, and the activity of α-amylase decreased in the pancreas. Kirchgessner and Schnegg (1980) suggested that the depression in digestion of starch by α-amylase might be indirectly responsible for their observed large depressions in the activities of hepatic enzymes and in the concentration of hepatic metabolites in nickel-deprived rats.

Anke *et al.* (1980) described the signs of nickel deprivation for goats and minipigs. The signs in these animals included depressed growth, delayed estrus, elevated perinatal mortality, unthriftiness characterized by a rough coat and scaly and crusty skin, depressed levels of calcium in the skeleton and of zinc in liver, hair, rib, and brain. Nickel-deprived goats also showed depressed hematocrit, hemoglobin, iron content in the liver, and triglycerides, β-lipoproteins, glutamate-oxaloacetate transaminase activity, and glutamate dehydrogenase activity in serum. The level of α-lipoproteins was elevated in serum.

Spears and Hatfield (1980) described nickel deprivation signs for sheep and cattle. Nickel-deprived lambs exhibited depressed growth, total serum protein, erythrocyte counts, ruminal urease activity, and total lipids, cholesterol, and copper in liver. Iron contents were elevated in liver, spleen, lung, and brain.

Signs of nickel deprivation in cattle fed a low-protein diet included depressed ruminal urease, serum urea, nitrogen, and growth.

Upon casual inspection, there appears to be some divergency in the described signs of nickel deprivation. Species differences might explain some disagreements, but, most likely, the iron status and age of the animals and the length of experiments probably were major determinants in the direction, extent, and severity of the signs of nickel deprivation obtained by various investigators. Taking the latter parameters into consideration, the signs of nickel deprivation agree relatively well. The major finding apparently is that nickel deprivation leads to abnormal iron metabolism.

In organisms other than animals, depressed growth is the predominant sign of nickel deprivation. Nickel is required for chemolithotropic growth of a number of hydrogen-oxidizing (Knallgas) bacteria (Tabillion *et al.*, 1980), including five strains of *Alcaligenes eutrophus*, two strains of *Xanthobacter autotrophicus*, *Pseudomonas flava*, *Arthrobacter* spec. 11X, and *Arthrobacter* strain 12x. Apparently, nickel is necessary for the synthesis of active soluble and membrane-bound hydrogenase in Knallgas bacteria. Growth of methanogenic bacteria, such as *Methanobacterium thermoautotrophism*, which utilize the formation of methane from H_2 and CO_2 as their energy source, is dependent on nickel (Thauer *et al.*, 1980). Depression in the formation of nickel-containing factor F_{430} *(vide supra)* may be responsible for the poor growth during nickel deprivation in these bacteria. Growth of microorganisms dependent on the nickel metalloenzyme urease is also depressed in nickel deprivation. These microorganisms include rumen bacteria (Spears and Hatfield, 1980) and urease-deficient mutant of *Aspergillus nidulans* (Mackay and Pateman, 1980). Nickel may also be required for growth of the Legionnaires' disease bacteria *Legionella pneumophilia* (Reeves *et al.*, 1981), a blue-gree algae (cyanobacteria) *Oscillatoria* sp. (Van Baalen and O'Donnell, 1978), and acetogenic bacteria such as *Clostridium thermoaceticium*, *C. formicoaceticium*, *C. aceticium*, and *Acetobacterium Woodii* (Thauer *et al.*, 1980). Carbon monoxide dehydrogenase activity apparently depends on nickel in the acetogenic bacteria that reduce CO_2 to acetate as their energy source.

The biological significance of nickel for plants was recently reviewed by Welch (1981). Nickel apparently is required by some plants for optimum growth, germination rate, and nitrogen utilization. Plants reported to require nickel for growth include *Chlorella vulgaris* (green algae), *Pinus radiata* (Monterey pine), and some nickel-accumulating species of *Alyssum*. Plant species that show improved growth upon nickel supplementation include wheat, grape vines, cotton, paprika, tomato, chinese hemp, potatoes, and soybean. Germination rates of seeds of peas, beans, wheat, castor beans, white lupine, soybeans, timothy, and rice apparently were improved by pretreating with, or germinating in the presence of a low concentration of, nickel. Plant growth dependent on urea-N, thus

dependent on the nickel-containing enzyme urease, is also depressed by nickel deprivation. With urea as the sole source of nitrogen, growth was depressed by nickel deprivation in plants such as duckweed (*Lemna paucicostata, Spirodela polyrrhiza,* and *Wolffia globosa*) and rice, tobacco, and soybean callus grown in suspension cultures. Most likely, any plant that requires urease in growth and metabolism would be adversely affected by nickel deprivation. Those plants and the point in their life cycle in which urease may be of importance are described by Welch (1981).

12.4 Nickel Function

To date, the most firmly established biological function of nickel is its *vide supra* described role in the enzyme urease. Thus, nickel is needed for the reaction:

$$(NH_2)_2CO + H_2O \xrightarrow[\text{urease}]{\text{Ni}} CO_2 + 2NH_3$$

Apparently, binding of the substrate urea to a nickel ion in urease is an integral part of the mechanism in the hydrolysis reaction. A carbamato-enzyme intermediate involving active-site nickel has been proposed. Nucleophilic attack or general base catalysis by a suitable active-site group would then lead to an active-site nickel–ammonia complex.

Although nickel is required for synthesis of active hydrogenases for the Knallgas reaction

$$2H_2 + O_2 \rightarrow 2H_2O$$

in Knallgas bacteria, the specific function in the synthesis is unknown. Nickel might be a constituent of both hydrogenase proteins or a regulatory protein essential at the transcriptional or translational level for the synthesis of the hydrogenases, or a factor needed to convert the hydrogenases into a catalytically active configuration (Friedrich *et al.*, 1981).

In acetogenic bacteria, the energy source reaction

$$8[H] + 2CO_2 \rightarrow CH_3COOH + 2H_2O$$

proceeds via formate, formyl tetrahydrofolate, methenyl tetrahydrofolate, methylene tetrahydrofolate, and methyl tetrahydrofolate. The reductive carboxylation of the methyl tetrahydrofolate to acetate is catalyzed by a multienzyme complex, part of which has carbon monoxide dehydrogenase activity. The synthesis of this part of the multienzyme complex is dependent on nickel. Most likely, nickel is a constituent of the carbon monoxide dehydrogenase (Thauer *et al.*, 1980).

In higher animals, the evidence showing that nickel is essential does not clearly define its metabolic function. The finding of nickel metalloenzymes in

plants and microorganisms suggests that a similar function for nickel in animals may be found. Nickel can activate many enzymes *in vitro,* but its role as a specific cofactor for any animal enzyme has not been shown. Other possible metabolic functions have been described (Nielsen, 1980b). Perhaps the most promising possibility is that nickel functions as a bioligand cofactor facilitating the intestinal absorption of the Fe^{3+} ion. This hypothesis is supported by findings from factorially arranged experiments which showed that nickel enhanced the absorption of iron present in the diet in less than adequate levels and in a relatively unavailable Fe^{3+} form. Nickel apparently had little or no effect on the absorption of the Fe^{2+} ion when it was present in the diet in less than adequate, but not severely inadequate levels. Possible mechanisms whereby nickel could enhance Fe^{3+} absorption were described by Nielsen (1980b).

12.5 Biological Interactions between Nickel and Other Trace Elements

In animals, plants, and microorganisms, nickel interacts with at least 13 essential minerals: Ca, Cr, Co, Cu, I, Fe, Mg, Mn, Mo, P, K, Na, and Zn. These interactions were recently reviewed by Nielsen (1980c). The interactions that appeared to be of most biological significance were those with iron, copper, and zinc.

The interaction between nickel and iron can be either synergistic or antagonistic. The synergistic interaction apparently occurs between nickel and ferric iron (Nielsen *et al.,* 1982). In factorially arranged experiments, hematopoiesis was affected by an interaction between iron and nickel when the iron supplement was ferric sulfate only. When the dietary ferric sulfate level was low, hemoglobin and hematocrit were lower in nickel-deprived than in nickel-supplemented rats. There was no evidence for an interaction when dietary iron was supplied as a mixture of ferric–ferrous sulfates. When the interaction between nickel and iron affected other parameters, such as the copper content of liver, it generally did so in a manner similar to that found with hemoglobin and hematocrit. That is, signs of nickel deprivation were more severe when dietary iron, as ferric sulfate, was low; or the signs of moderate iron deficiency were more severe when dietary nickel was deficient.

The antagonistic interaction apparently occurs between nickel and ferrous iron (Nielsen *et al.,* 1982). Severe iron deficiency was more detrimental to nickel-supplemented than to nickel-deficient rats, as growth was more severely depressed and perinatal mortality was higher in nickel-supplemented rats. This suggests nickel impaired the utilization of the small amount of dietary iron apparently in the ferrous form.

The form of dietary iron also apparently affects the nature of the signs of nickel deficiency. When only ferric sulfate was supplemented to the diet, plasma

and liver total lipids were elevated, and liver iron content was depressed in nickel-deprived rats. On the other hand, when a ferric–ferrous mixture was supplemented to the diet, nickel deprivation depressed plasma total lipids did not affect liver total lipids and elevated the liver content of iron.

Most of the interaction between nickel and iron probably occurs during absorption. There is evidence that both active and passive transport mechanisms play a role in iron absorption. Active transport of iron to the serosal surface is relatively specific for the divalent cation so the ferric ion is absorbed by passive transport. Becker *et al.* (1980) reported that the transport of nickel across the mucosal epithelium apparently is an energy-driven process rather than simple diffusion and suggested that nickel ions use the iron transport system. Because different mechanisms are involved in the absorption of the two forms of iron, and nickel is involved in both mechanisms, it seems likely that iron nutrition should affect nickel absorption and requirement, and vice versa. Evidence for the competitive interaction between nickel and iron during absorption includes the findings that the transfer of nickel from the mucosal to the serosal side of iron-deficient rat intestinal segments was elevated (Becker *et al.*, 1980), and that moderately anemic iron-deficient rats absorbed approximately 2.5 times as much $^{63}Ni^{2+}$ administered via gavage, as did those fed a control diet (Ragan, 1978). Thus, in nickel deficiency, because of the lack of competition with the Ni^{2+} ion, more of the active transport system could be utilized for iron absorption. This would explain the finding of an increased iron content in liver of nickel-deprived rats fed dietary iron in the ferrous form. On the other hand, nickel apparently promotes the passive transport of Fe^{3+} because in nickel deficiency the absorption of Fe^{3+} is depressed. This would explain the finding of a decreased iron content in liver of nickel-deprived rats fed dietary iron as ferric sulfate only. These changes in iron absorption, leading to changes in levels of iron and other trace elements in tissue, probably explain the apparent divergent effects of nickel deprivation on plasma and liver total lipids. In other words, dietary nickel affects these parameters indirectly, with the direction and magnitude of the changes determined by the extent and the mechanism through which nickel and iron interact during their absorption.

The competitive interaction between nickel and iron is possible because they both can form the same type of complex. Nickel ions can possess outer orbital bonding in which the coordination number is 6 and form octahedral complexes. Both ions of iron (Fe^{2+}, Fe^{3+}) can have a coordination number of 6 and form octahedral complexes regardless of whether there is inner or outer orbital bonding. Thus, the same orbitals could be involved in bonding both nickel and iron when they form outer orbital complexes.

Cuprous and cupric ions have a preferred coordination number of 4 and form tetrahedral and square coplanar complexes, respectively. Nickel can possess

inner orbital bonding, in which the preferred coordination number is 4, and form either tetrahedral or square coplanar complexes. Thus, if nickel is present in biological systems with a coordination number of 4, nickel and copper could have similar chemical parameters and a nickel–copper interaction probably would be competitive. Supporting this suggestion are findings from factorially arranged experiments that show an antagonistic interaction between nickel and copper (Nielsen *et al.*, 1982). In rats deficient in copper, with significant but not too severe anemia, the copper deficiency signs of elevated heart weight and plasma cholesterol, and depressed hemoglobin, were exacerbated by nickel supplementation. The effect was greater when dietary nickel was 50 μg/g rather than 5 μg/g.

Nickel supplementation did not depress the level of copper in liver, or plasma, of copper-deficient rats. This finding indicates that nickel did not exacerbate copper deficiency signs by interfering with the absorption of copper. The antagonism between copper and nickel was probably due to the isomorphous replacement of copper by nickel at various functional sites. If nickel did not perform, or less efficiently performed, the functions of copper, the end result would be less copper function at various physiological sites and a more severe copper deficiency.

Zn^{2+} has a preferred coordination number of 4 and forms tetrahedral complexes. As mentioned *vide supra,* nickel ions can have inner orbital bonding, in which the preferred coordination number is 4, and form either tetrahedral or square coplanar complexes. Thus if nickel forms tetrahedral complexes in biological systems, a competitive interaction between zinc and nickel should occur. However, to date, most of the findings indicate that the interaction between nickel and zinc is noncompetitive. Instead of interacting directly at sites of zinc function, deficient or toxic levels of nickel apparently act indirectly to shift slightly the distribution of zinc in the body. For example, Anke *et al.* (1980) found that nickel deprivation depressed the zinc content of liver, hair, rib, and brain in minipigs and goats. Because some signs of nickel deprivation were similar to those of zinc deficiency, Anke *et al.* (1980, 1981) suggested nickel deficiency disturbs zinc metabolism. Kirchgessner and Pallauf (1973) found that depressed growth and other signs of zinc deficiency in the rat were not changed by supplementation with 50 μg of nickel/g of diet. Nickel supplementation did reduce serum zinc and increased the zinc concentration in the liver. Spears *et al.* (1978) found that in the rat 50 μg of nickel/g of diet partially alleviated some signs of zinc deficiency, but did not affect others. They noted that nickel depressed tibia zinc but elevated liver zinc in the zinc-deficient animals. This suggests that nickel, through an indirect means, redistributed zinc in the animal body so that some signs of deficiency were alleviated.

Because nickel competitively interacts with copper and iron, but apparently

not with zinc, nickel probably forms square coplanar or octahedral complexes in biological systems. The formation of tetrahedral complexes by nickel *in vivo* probably occurs infrequently.

12.6 Nickel Metabolism and Toxicity

In higher animals there are four entry routes into the body—oral intake, inhalation, percutaneous absorption, and parenteral administration. In metabolism, oral intake is of primary importance, and thus is emphasized here. Parenteral administration of nickel, at present, is used only to study nickel distribution, metabolism, and toxicity, and will be discussed only when it indicates possible mechanisms in handling oral nickel. Percutaneous absorption and inhalation entry routes are of major importance in nickel toxicity.

Most ingested nickel remains unabsorbed by the gastrointestinal tract and is excreted in the feces (Sunderman, 1977). Some fecal nickel may come from the bile as nickel has been found in the bile of rats and rabbits injected with ^{63}Ni(II). Limited studies indicate that less than 10% of ingested nickel is normally absorbed. However, a higher percentage may be absorbed during gravidity (Kirchgessner *et al.*, 1981). As mentioned *vide supra,* the transport of nickel across the mucosal epithelium appears to be an energy-driven process rather than simple diffusion. Nickel ions apparently use the iron transport system located in the proximal part of the small intestine.

Although fecal nickel excretion is 10–100 times as great as urinary excretion, the small fraction of nickel absorbed from the intestine and transported to the plasma is excreted primarily via the urine as low-molecular complexes believed to include histidine and aspartic acid. Sweat may also be important in nickel metabolism because in healthy adults it contains several times the amount found in serum. This suggests an active secretion of nickel by sweat glands. However, excretion of nickel by sweat apparently is unresponsive to acute elevated doses of oral nickel (Christensen *et al.*, 1979). Thus, measurements of serum and urinary, but not sweat, nickel may be used to detect variations in the oral intake of nickel.

Transport of nickel in blood is accomplished by serum albumin and by ultrafilterable serum amino acid ligands. No tissue except possibly fetal tissue significantly accumulates nickel. The kinetics of $^{63}Ni^{2+}$ metabolism in rodents apparently fits a two-component model. A summary of the tissue retention and clearance of $^{63}Ni^{2+}$ administered by all routes of entry has been given by Kaspizak and Sunderman (1979). This summary shows that kidney retains significant levels of nickel shortly after $^{63}Ni^{2+}$ is given. The retention probably reflects the role of the kidney in nickel excretion. The level of $^{63}Ni^{2+}$ in kidney falls quickly

over time. Also, studies with $^{63}Ni^{2+}$ show that nickel readily passes through the placenta. Embryonic tissue retains greater amounts of parenteral administered nickel than does that of the dam (Jacobsen *et al.*, 1978). Also, amniotic fluid retains relatively high amounts of orally administered nickel (Kirchgessner *et al.*, 1981). The level of nickel in the fetus does not fall quickly after parenteral administration to the dam, thus suggesting retention or inhibited clearance by the fetus.

The preceding discussion shows that there are mechanisms for the homeostatic regulation of nickel. Thus, it is not surprising that life-threatening toxicity of nickel through oral intake is low, ranking with such elements as zinc, chromium, and manganese. Nickel salts exert their toxic action mainly by gastrointestinal irritation and not by inherent toxicity. Large oral doses of nickel salts are necessary to overcome the homeostatic control of nickel. Generally, 250 μg or more of nickel/g of diet is required to produce signs of nickel toxicity in rats, mice, chicks, rabbits, and monkeys (Nielsen, 1977). The ratio of the minimum toxic dose and the minimum dietary requirement for chicks and rats is apparently near 5000. If animal data can be extrapolated to humans, this translates into a daily dose of 250 mg of soluble nickel to produce toxic symptoms in humans.

Recent findings, however, suggest that oral nickel in not particularly high doses can adversely affect health under certain conditions. The effects of relatively low levels of dietary nickel on copper deficiency and severe iron deficiency were described *vide supra*. Another important condition occurs in humans with an allergy to nickel. Finally, the tendency of the fetus to retain nickel suggests that elevated levels of nickel in the blood should be avoided during pregnancy.

Nickel dermatitis is a relatively common form of nickel toxicity in humans. Several surveys have shown that incidence of sensitivity to nickel is between 4 and 13% (Nielsen, 1977). Until recently, nickel dermatitis has been thought to be caused mainly by the percutaneous absorption of nickel. However, Christensen and Möller (1975) presented evidence that suggested the ingestion of small amounts of nickel may be of greater importance than external contacts in maintaining hand eczema. Cronin *et al.* (1980) observed that an oral dose of 0.6 mg of nickel as nickel sulfate ($NiSO_4$) produced a positive reaction in some nickel-sensitive individuals. That dose is only 12 times as high as the human daily requirement postulated from animal studies.

Lu *et al.* (1981) found that the injection of $NiCl_2$ intraperitoneally in mice caused teratology. They found also that the kinetics of nickel chloride in fetal tissues was different from that in maternal tissues and suggested anomalies caused by elevated nickel levels could occur in embryo without recognizable adverse effects in maternal mice.

The metabolism and toxicity of the carcinogen nickel carbonyl differs markedly from that of Ni^{2+}. Nickel carbonyl is highly volatile and is absorbed readily

by the lungs. The inhalation route is the most important in respect to nickel carbonyl toxicity. Also, the metabolism and toxicity of relatively insoluble nickel compounds such as Ni_3S_2 (usually administered intramuscularly or intrarenally) differ from Ni^{2+} in their metabolism. A review on the toxicity of nickel carbonyl and relatively insoluble nickel compounds was given by Sunderman (1977), and thus, will not be done here.

12.7 Summary

Interest in the biochemistry of nickel has been stimulated by recent discoveries of its essentiality to various microorganisms, plants, and animals and of the existence of several nickel metalloenzymes in plants and microorganisms. Signs of nickel deprivation have been described for six animal species—chick, cow, goat, minipig, rat, and sheep. Included among the more consistent signs of deficiency in mammals are depressed growth, unthriftiness characterized by rough hair coat, and an altered iron metabolism leading to depressed hematopoiesis. The predominant sign of nickel deficiency for microorganisms is depressed growth, and for plants is depressed nitrogen utilization. In plants and microorganisms, nickel is known to function in several metalloenzymes including urease, several hydrogenases, and carbon monoxide dehydrogenase. In higher animals, the evidence showing that nickel is essential has not defined its metabolic function. The finding of nickel metalloenzymes in lower forms of life suggests that a similar function for nickel in animals may be found.

Divalent nickel is the apparent important oxidation state in the metabolism of nickel. Ultrafilterable Ni^{2+} binding ligands, perhaps histidine and cystine, apparently play important roles in the extracellular transport of nickel, intracellular binding of nickel, and excretion of nickel in urine and bile. Two Ni^{2+} binding proteins suggested to have a role in the transport and homeostasis of nickel in serum are albumin and histidine-rich glycoprotein. The transport of nickel across the mucosal epithelium apparently occurs as Ni^{2+} and is an energy-driven process, rather than one driven by simple diffusion, and is probably connected with the iron-transport system. Only recently another oxidation state of nickel has been indicated to be important in biochemistry. Ni^{3+} apparently is important for the activity of bacterial hydrogenases.

In conclusion, emerging evidence indicates that nickel is a dynamic trace element in living organisms. However, knowledge of the biochemistry of nickel is very limited. Thus, further research on nickel biochemistry is needed to help evaluate the nature and importance of the physiologic, pharmacologic, and toxicologic actions of nickel.

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